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Not to be lost in translation

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Published in:
Clinical and Translational Immunology

DOI:
[10.1038/cti.2017.51](https://doi.org/10.1038/cti.2017.51)

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
Pociot, F. (2017). Type 1 diabetes genome-wide association studies: Not to be lost in translation. *Clinical and Translational Immunology*, 6(12), [e162]. <https://doi.org/10.1038/cti.2017.51>

REVIEW

Type 1 diabetes genome-wide association studies: not to be lost in translation

Flemming Pociot^{1,2,3}

Genetic studies have identified >60 loci associated with the risk of developing type 1 diabetes (T1D). The vast majority of these are identified by genome-wide association studies (GWAS) using large case-control cohorts of European ancestry. More than 80% of the heritability of T1D can be explained by GWAS data in this population group. However, with few exceptions, their individual contribution to T1D risk is low and understanding their function in disease biology remains a huge challenge. GWAS on its own does not inform us in detail on disease mechanisms, but the combination of GWAS data with other omics-data is beginning to advance our understanding of T1D etiology and pathogenesis. Current knowledge supports the notion that genetic variation in both pancreatic β cells and in immune cells is central in mediating T1D risk. Advances, perspectives and limitations of GWAS are discussed in this review.

Clinical & Translational Immunology (2017) 6, e162; doi:10.1038/cti.2017.51; published online 1 December 2017

Type 1 diabetes (T1D) is a chronic immune-mediated disease causing attrition and death of the insulin-producing pancreatic β cells, resulting in a life-long requirement for exogenous insulin. The progressive loss of β cells is mainly owing to autoimmune inflammation.¹ Worldwide >20 million people are afflicted with T1D. By 2015, more than half a million children are estimated to be living with T1D and ~86 000 children develop T1D each year (www.diabetesatlas.org). In most countries T1D incidence is increasing by ~3–4% every year, most notably in children and adolescents.² Five million people in the USA are expected to have T1D by 2050, including ~600 000 youth.^{3,4} Existing treatments do not relieve the disease burden, for example, severe hypoglycemia is common^{5,6} and >70% of patients are unable to maintain a healthy HbA1c.^{7,8} Life-expectancy is reduced by up to 13 years; even with good HbA1c control, life expectancy is reduced by ~8 years.⁹

Fifteen percent of newly diagnosed T1D patients have a first-degree family member with T1D. The T1D concordance rates are in the range of 30–70% in monozygotic twins and 3–13% in dizygotic twins.^{10–12} This non-Mendelian inheritance pattern is characteristic for multifactorial diseases and results from the contribution of several genes each having only a minor influence on disease development.¹³ In addition to genetic predisposition, environmental and epigenetic factors impact the disease susceptibility.^{14,15}

In this review, only studies of genetic architecture of T1D are discussed with focus on the translation of genetics into biology. For the last two decades, genome-wide approaches to map the genetic risk have been prevailing. First, as linkage studies using affected sib-pairs and subsequently as genome-wide association studies (GWAS) using a case-control design. Whereas the initial genome-wide linkage studies

(GWLS) mainly confirmed established associations (*HLA*, *INS* and *CTLA4*) from previous candidate-gene studies,^{16–20} more recent and larger GWLS also provided novel information on the genetic predisposition to T1D.^{21–23} Nevertheless, very few of the novel loci identified from these 1st, 2nd and 3rd generation GWLS have been replicated and confirmed in more recent GWAS data sets. This is mainly owing to the inherited limitations in GWLS, which include limited power to narrow down risk variants and to detect risk variants with only minor contribution. An example of a novel region associated with T1D identified by GWLS and subsequently confirmed by GWAS, is the *UBASH3A* region on chromosome 21.²⁴

T1D GENETICS—THE PRE-GWAS ERA

Genetic studies have had an essential role in understanding T1D biology.²⁵ The first reports of genetic association to T1D were for the human leukocyte antigen (HLA) region.^{26–28} As this discovery, researchers have tried to understand the underlying mechanisms by which alleles of HLA-encoding genes are responsible for the T1D association. Although much has been learned about the effects of certain HLA alleles on T1D risk, the exact biological mechanism of HLA-conferred susceptibility remains elusive. The extreme polymorphism of the HLA locus makes association analyses complicated. In addition, the strong linkage disequilibrium in the region makes assessment of individual risk variants challenging. The HLA region is the most polymorphic observed in the human genome, with 17 166 unique alleles reported as of July 2017 (<http://www.ebi.ac.uk/imgt/hla/stats.html>). The genetic risk for T1D in Caucasians is conferred mainly by combinations of HLA-DR and -DQ genes, for example, those encoding DR4-DQ8 (that is, *DRB1*04*, *DQA1*03*-*DQB1*03:02*) and

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Received 30 August 2017; revised 15 October 2017; accepted 16 October 2017

Table 1 Type 1 diabetes genome-wide association studies (GWAS) reported in the GWAS Catalogue (www.ebi.ac.uk/gwas)

Study	Sample description (cases/ controls)	Replication sample (cases/ controls)	Platform (SNPs passing QC)	Significant associations ($P \leq 5 \times 10^{-8}$)
WTCCC ³⁶	1963/2938		Affymetrix (469 557)	10
Todd <i>et al.</i> ¹⁰²	2000/3000	4000/50 002 997 trios	Affymetrix (NR) ^a	12
Hakonarson <i>et al.</i> ¹⁰³	561/1 143 467 trios	1333/390 trios	Illumina (543 071)	4
Cooper <i>et al.</i> ¹⁰⁴	3561/4646	6225/69 463 064 trios	Affymetrix (335 565)	14
Hakonarson <i>et al.</i> ¹⁰⁵	561/1 143 467 trios	946/1 098 364 trios	Illumina (543 071)	1
Barrett <i>et al.</i> ⁸⁷	7514/9045	4267/4 6704 342 trios	Affymetrix/Illumina (841 622 (imputed))	38
Grant <i>et al.</i> ¹⁰⁶	563/1 146 483 trios	3303/4673	Illumina (1 000 000)	5
Wallace <i>et al.</i> ⁷⁴	7514/9045	4840/26 705 766 trios	Affymetrix/Illumina (2 600 000 (imputed))	2
Bradfield <i>et al.</i> ¹⁰⁷	9934/16 956	1120 trios	Affymetrix/Illumina (2 540 000 (imputed))	9
Huang <i>et al.</i> ¹⁰⁸	16 179 ^b		(6 233 112) (imputed)	2
Onengut-Gumuscu. ^{c39}	6808/128 352 601 ASP 69 trios		ImmunoChip (138 229)	44 ^d

The main type 1 diabetes genome-wide association studies listed with sample sizes for initial discovery and replication, analysis platform and number of significant observations.

^aNot reported.

^bThis study used 1000 Genomes-based imputation to identify associations from the Wellcome Trust Case Control Consortium phase 1 Data.³⁶

^cThis study is a fine mapping study using the ImmunoChip.⁶⁷

^d $P \leq 3.23 \times 10^{-7}$ (ImmunoChip Bonferroni-corrected $P < 0.05$).

DR3-DQ2 (that is, *DRB1*03*, *DQA1*05-DQB1*02*), and particularly those present in HLA-DQ2/DQ8 heterozygotes are associated with high susceptibility to T1D. In contrast, a particular DQ6 molecule, encoded by *HLA-DQA1*01:02-DQB1*06:02*, is associated with strong protection from the disease, even in the presence of high-risk HLA alleles and/or T1D-associated autoantibodies.²⁹ Today, >60 loci associated with T1D have been identified. However, the HLA association remains the strongest by far, with reported odds ratios ranging from 0.02 to >11 for specific DR-DQ haplotypes.³⁰ After HLA, the strongest T1D genetic association comes from polymorphism in the promoter region of the insulin gene (odds ratios=2.4).¹³ Only two other loci, *PTPN22* and *IL2RA*, have consistently reported odds ratios greater than 1.5; most others are in the range of 1.05–1.25 underscoring the importance of the HLA region compared with other loci.¹³ Thus, all studies of T1D genetic susceptibility should take HLA into account when interpreting association data for any other candidate loci.³¹

GWAS STUDIES

During the past decade, GWA studies have represented a paradigm shift in strategies for identifying risk genes for complex (multifactorial) human diseases, including T1D. In GWAS a large number (up to millions) of variants are tested in a hypothesis-free context. The first successful GWAS was published in 2005. It investigated patients with age-related macular degeneration and found two SNPs with significantly altered allele frequency compared to healthy controls.³² Today, >3000 GWAS publications are catalogued by the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI).³³ The Catalog is a quality controlled, manually curated and literature-derived collection of all published GWAS assaying at least 100 000 SNPs and all SNP-trait associations with P -values $< 1.0 \times 10^{-5}$ are reported.³⁴ The GWAS Catalogue reports 64 SNP-trait associations for T1D, but notably the disease-causing variants and genes are still largely unknown. The leading role in these studies belongs to International Consortia, which possess individual DNA samples from various cohorts; among the main leaders have been the T1DGC (International Type 1 Diabetes Genetics

Consortium),³⁵ and the WTCCC (Wellcome Trust Case Control Consortium).³⁶ Noteworthy, GWAS do not necessarily identify the specific gene or genes in a given locus responsible for the observed disease association, and do not typically inform the wider context in which the disease genes operate.^{37,38} Thus, GWAS on their own provide limited insights into the molecular mechanisms driving disease. Numerous GWAS have been performed in T1D, Table 1, and identified several genomic regions associated with increased risk to T1D. There is a significant overlap in study populations between the studies, for example, the WTCCC-, T1DGC-, as well as other cohorts are included in several of the GWAS. Notably, all study populations were of European ancestry.

The principle has been to 'name' these GWAS regions after the gene closest to the strongest associated marker (the lead SNP) or, alternatively, after gene(s) with (some) biological significance for the disease pathology. However, almost all T1D GWAS-associated regions contain multiple genes and thorough fine mapping is therefore essential to narrow down the causal variant(s). The most extensive fine mapping has been performed by the ImmunoChip study,³⁹ which was designed to make genetic comparisons across autoimmune disorders as informative as possible. The ImmunoChip genotyping confirmed and narrowed down most GWAS identified risk loci and also identified new T1D-associated regions ($P < 5 \times 10^{-8}$).

The heritability obtained from twin and sibling studies ranges from 0.4 to 0.92.^{10,12,40–42} That is, assuming the heritability estimates are correct. This may not always be the case as GWAS have been performed in outbred populations with little evidence of familial clustering of the disease. It is thus quite possible that we have overestimated heritability, which often is inferred from twin or family studies. The familial clustering of T1D, in contrast to most other complex diseases, can be explained almost completely by the multiple common variants identified by GWAS. The estimated proportion of heritability explained by currently identified loci is >80%.⁴³

As most variants identified through GWAS contribute to only modest effects to disease risk, it is likely that a combination of variants will better capture effects of clinical relevance. The impact of multiple variants on disease prediction and progression has been evaluated in

candidate gene-based studies,^{44–46} and more recently using a genome-wide approach.^{47,48} Taken together these studies demonstrate that combining information from several loci in a T1D genetic risk score accurately can identify young adults with diabetes.⁴⁷

PREDICTION OF LIKELY TARGETS

To identify the underlying causal disease mechanisms it is important to prioritize among the many GWAS signals. This is often done by integration of other data sets. A common initial step is to overlap genomic features, such as expression quantitative trait loci (eQTLs), transcription factor-binding sites, DNase hypersensitive sites and histone modifications, with SNP position.⁴⁹ This is considered a biologically plausible approach and has provided important insight—especially from the combination of genetic data with expression data. SNPs that influence gene expression are called expression quantitative trait loci (eQTL), and it has been demonstrated that complex trait-loci are enriched for eQTLs.⁵⁰

This appears to be the case also for T1D.⁵¹ A potential limitation is that eQTLs often are tissue specific and studies have often used lymphoblastoid cell lines as a proxy for the autoimmune process in T1D. However, even between immune cell populations eQTLs vary.^{51,52} Non-synonymous SNPs change amino-acid composition or truncate the protein sequence by introducing a stop-codon. Synonymous SNPs may affect splicing sites resulting in alternative mRNA isoforms. Structured variations as indels may have the same consequences. Although several non-synonymous SNPs have been identified in T1D risk genes, these missense SNPs are as such not enriched in T1D loci and have, with the exceptions of *HLA* and *PTPN22*, not been convincingly demonstrated to be causal. Identification of differential gene expression profiles in T1D cases and control subjects or in T1D model systems may suggest disease mechanisms/pathways for follow-

up studies.^{37,53,54} Finally, it was recently demonstrated that GWAS loci, including T1D loci, were enriched for SNPs mapping to regulatory element.^{55,56} Thus, several approaches are used for prioritization of risk variants/genes for follow-up studies. In addition, there are several computational tools available for prioritizing SNPs for further downstream analysis (for example, see <https://omictools.com/snp-prioritization-category>).

TARGET GENES SUBSEQUENTLY FOUND BY FUNCTIONAL STUDIES TO INFLUENCE PATHOPHYSIOLOGY

Determining the mechanisms of action of T1D risk variants is challenging, owing to interaction effects, cell type-specific gene expression, the local tissue milieu, the temporal course of gene expression and complicating environmental factors. Great efforts over the last few years have highlighted potentially functionally target genes that influence T1D pathophysiology. These have mainly been studied in β cells or immune cells. In T1D, the pancreatic β cells in the islets of Langerhans are selectively destroyed by the immune system resulting in absolute insulin deficiency. At least 40% of the genes in the T1D susceptibility loci are expressed in human islets and β cells, where they according to recent studies modulate the β -cell response to the immune system.^{37,53,57–62} At least half of these are regulated by cytokines *in vitro*,^{37,53,57} an often used model system for T1D pathogenesis. To explore causality of these gene variants in β -cells, functional studies in experimental models, for example, knockdown and overexpression studies, is necessary. Furthermore, studies on knock-in and knock-out mice will, in many cases, aid in the understanding of how the candidate genes affect the disease pathogenesis and contribute to the risk of T1D. This field is in its early stages, but recent studies (reviewed in^{58–62}) have identified candidate genes that affect β -cell function or survival in T1D settings, Table 2.

Functional studies of immune cells support the potential impact of T1D risk variants on gene regulation in immune cells.^{51,63,64} *HLA* has a pivotal role as antigen-presenting molecules and as such in the autoimmune process. For non-*HLA* T1D SNPs strongest evidence comes from their role in regulating gene expression. A recent review found close to 100 eQTLs in different immune cells for T1D risk SNPs.⁶³ Noteworthy, eQTLs vary between different immune cells, for example, between $CD4^+$ and $CD8^+$ T cells, underlying the importance of studying fractionated immune cell populations. Several studies point to delineation of regulatory or effector pathways as autoreactive $CD4^+$ T cells as key immunological mechanisms affected by T1D genetic risk.^{65,66}

NEW INSIGHTS ARISING FROM GWAS

The wealth of data generated by GWAS has also informed our biological understanding of disease processes. This comes from, for example, identification of pleiotropic risk loci, by assessing both disease susceptibility and protection, and by studying the potential role of non-coding RNAs.

Overlapping etiological factors in autoimmune diseases have been recognized for a long time due shared clinical and immunological features. Also, T1D share genetic susceptibility loci with a number of other IMD (immune-mediated diseases). This has been recognized for the *HLA* region for a long time and more recently GWAS studies have added a considerable number of pleiotropic susceptibility loci, that is, SNPs that confer susceptibility to more than one IMD (reviewed in⁶⁷). Interestingly, T1D loci primarily show concordant overlap, that is, same SNP allele confer risk, with other seropositive autoimmune diseases (for example, autoimmune thyroid disease, rheumatoid arthritis, celiac disease), whereas discordant association, that is, same

Table 2 Candidate genes affecting β -cell functions

Gene (Chromosome)	Variant(s)	Function/pathway affected	Reference
INS (11p15.5)	INS VNTR class I rs7111341	β -cell expression level	70,71
	rs11564705 ^a		
IFIH1 (2q24.2)	rs1990760 rs3747517	MDA5 signalling	73
GLIS3 (9p24.2)	rs7020673	β -cell development	109,110
		β -cell apoptosis	
		GLUT2 expression	
PTPN2 (18p11.21)	rs1893217 rs2542151 ^a	Inflammation and virus-induced β -cell apoptosis	111–113
CTSH (15q25.1)	rs3825932 rs11856301 ^a	Cytokine-induced apoptosis	99
		Insulin transcription	
BACH2 (6q15)	rs11755527	Cytokine-induced apoptosis	114
TYK2 (19p13.2)	rs2304256	Inflammation and virus-induced β -cell apoptosis	75
CLEC16A (16p13.13)	rs12444268 rs12708716 rs11865121 ^a	Autophagy/mitophagy Insulin secretion	115,116

Based on ⁸⁵ is listed.

^aT1D candidate genes where experimental studies support their functional significance in β cells. Adapted from^{58,62} variant(s) shows the lead SNP identified in GWAS and in cases where the lead SNP is not the most likely one to be functional then the potentially functionally SNP.

Table 3 Examples of pleiotropic non-HLA loci in type 1 diabetes and other immune-mediated diseases

IMD	Loci
Alopecia areata	1p13.2, 11q13.1, 12q13.2, 12q24.12
Autoimmune thyroid disease	1p13.2, 2q33.2, 6q15
Celiac disease	2q33.2, 3p21.31, 4q27, 6q15, 6q23.3, 6q25.3, 12q24.12, 15q25.1, 18p11.21, 18q22.2
Crohn's disease	1p13.2, 1q32.1, 11q13.1, 16p11.2, 18p11.21, 19p13.2, 19q13.33
Inflammatory bowel disease	1q32.1, 2q24.2, 6q23.3, 18p11.21, 19q13.33
Juvenile rheumatoid arthritis	1p13.2, 12q24.12, 19p13.2
Multiple sclerosis	6q15, 6q25.3, 11q13.1, 12p13.31, 12q14.1, 16p13.13, 18q22.2, 19p13.2
Narcolepsy	15q25.1
Primary biliary cirrhosis	2q32.3, 6q23.3, 11q13.1, 12q24.12, 16p13.13, 17q12, 17q21.31, 19p13.2
Primary sclerosing cholangitis	12q24.12, 14q24.1
Psoriasis	2q24.2, 19p13.2
Rheumatoid arthritis	1p13.2, 2q11.2, 2q32.3, 2q33.2, 4p15.2, 6q15, 6q23.3, 10p15.1, 12q24.12, 17q12, 19p13.2, 21q22.3
Systemic lupus erythematosus	1p13.2, 1q32.1, 2q32.3, 6q23.3
Ulcerative colitis	1q32.1, 2q24.2, 4q27, 6q23.3, 18p11.21
Vitiligo	1p13.2, 2q24.2, 10p15.1, 12q24.12, 21q22.3

Abbreviations: AA, alopecia areata, ATD, autoimmune thyroid disease, CEL, celiac disease, CRO, Crohn's disease, IBD, inflammatory bowel disease, JRA, juvenile rheumatoid arthritis, MS, multiple sclerosis, NAR, narcolepsy, PBC, primary biliary cirrhosis, PSC, primary sclerosing cholangitis, PSO, psoriasis, RA, rheumatoid arthritis, SLE, Systemic lupus erythematosus, T1D, type 1 diabetes, UC, ulcerative colitis, VIT, vitiligo. Approximately half of the type 1 diabetes peak SNPs show association with another disease. Adapted from¹⁷

SNP allele shows risk in opposite directions, is more common to seronegative IMD, Table 3. The study of these pleiotropic risk variant holds great potentials for unraveling the functional significance of these variants.

It has been acknowledged for many years that whereas specific HLA haplotypes confer the strongest genetic risk for T1D other HLA haplotypes provide protection against T1D. The DR2-DQ6 (*DQB1*06:02*) haplotype is strongly protective against T1D²⁹ and in the rare T1D patients that are *DQB1*06:02* positive, the autoimmune process appears to be unique.⁶⁸ Although the exact underlying mechanism(s) for the observed HLA susceptibility is (are) not fully understood, the basic function of HLA molecules, that is, peptide presentation, is compliant with the observation of both predisposing and protective HLA molecules in T1D risk. The second T1D susceptibility gene identified in the pre-GWAS era, the insulin gene (*INS*), also has both predisposing and protective variants.⁶⁹ In this case, the effect is ascribed to tissue specificity and expression level.^{70,71}

Interestingly, fine mapping studies of GWAS-identified candidate genes have demonstrated that in some cases rare variants with opposite effect exist. For example, GWAS-identified non-synonymous variants (rs1990760 and rs3747517) in the *IFIH1* locus on 2q24.2 primarily mediate susceptibility through increased type I interferon production.⁷² In addition to these common SNPs, Nejentsev *et al.*⁷³ reported the presence of four rare SNPs associated with T1D (rs35667974, rs35337543, rs35732034, rs35744605). These rare alleles are associated with protection from T1D in contrast to the common SNPs.⁷³ The location of T1D-associated SNPs within the *IFIH1* sequence suggests that these point mutations are either negatively or positively affecting the *IFIH1*-encoded protein MDA5 signaling via a mechanism involving the C-terminal end of the gene.⁷³ Tyrosine Kinase 2 (*TYK2*) is located on 19p13.2 and harbors a non-synonymous SNP (rs2304256) that causes a missense mutation in *TYK2* associated with a lower risk of T1D.⁷⁴ This is most likely caused by changes in *TYK2* expression that dampens the type I interferon response in virus-infected β cells.⁷⁵ However, reduced *TYK2* expression caused by rare promoter mutations predispose to virus-induced diabetes in rodents⁷⁶ and in the Japanese population.⁷⁷ Thus, interpretation of association signals from GWAS should be cautious,

even when fine mapping has narrowed it down to a single candidate gene.

Therefore, to fully understand disease pathogenesis from GWAS, it is important to analyze the data in the context of complementary datasets, such as transcriptomics, metabolomics, proteomics, under conditions relevant for the disease. It has been advocated that analysis at the pathway, network or protein complex level is the next step in the process of GWAS data mining.⁷⁸ Present evidence suggests genetic risk variants for T1D are organized in pathways, physically interact with one another, and are enriched for protein–protein interaction network modules.^{37,79,80}

To analyze high-throughput data different data filtering approaches are often taken. Intrinsic data filtering uses information from the dataset itself, such as filtering genetic variants based on linkage to other variants of interest in that data set. Extrinsic data filtering is based on information outside of the data set, such as the inclusion of genomic annotations from separate studies such as ENCODE. At present, both intrinsic and extrinsic data filtering are essential for efficient characterization of T1D genetics. Often multistage and meta-dimensional analysis approaches are used to explore relationships between data sets. In brief, multistage analysis sequentially examines relationships between each data set, and also between each data set and the trait, for example, as the analysis of eQTLs, which includes analysis of genetic variants and gene expression levels. Meta-dimensional analysis takes advantage of simultaneous combination of multiple data types into a single search space to construct a final model. This form of analysis may use several types of data modelling and integration strategies toward the final analysis.⁸¹ Clearly, there is a need for further developing systems biology approaches to provide new insight on T1D biology. We recently used such an approach to construct protein interaction networks from all genes located in non-HLA loci associated with T1D combined with tissue-specific transcriptomic data to identify significantly regulated network.^{37,82–85} This indicates that protein networks can add biological context to candidate genes identified through GWAS.

Interestingly, >90% of disease-associated SNPs map within the non-coding regions of the genome such as promoters, enhancers, intergenic regions and ncRNA genes,⁸² suggesting a regulatory role. This supports the concept that changes in regulation of gene

expression are a key factor in T1D development. Our understanding of the modes of action of most ncRNAs, excluding miRNAs, is still very rudimentary.⁸³ It is a major challenge to develop tools and models that will capture the function of ncRNAs, especially the longer ones, where function is executed by structured elements rather than defined by linear sequences.⁸⁴ Nevertheless, it is foreseen that a more in-depth understanding of ncRNAs may in near future open new strategies for diagnostics, classification and personalized therapeutic regimens in T1D and other immune-mediated diseases.⁸⁵

LIMITATIONS OF PUBLISHED GWAS, OTHER CHALLENGES AND PRIORITIES IN THE FIELD

Although GWAS have a range of potentials as discussed above there is a number of limitations and challenges as well. Missing heritability owing to the undiscovered effect of rare variants and epistasis are two key concerns.⁸⁶ GWAS studies predominantly test for association of common SNPs, that is, with minor allele frequency > 5%. Rare variants do not seem to account for a major part of T1D susceptibility, though it has been demonstrated that rare variant in, for example, *IFIH1* affect T1D risk.⁷³ Epistasis is difficult to test properly, but most studies suggest that this is not a major player in T1D predisposition.^{20,87,88} However, it is possible that many GWAS SNPs having low or moderate risk in themselves, that is, *P*-values just above 5×10^{-8} , interact to confer a significant combined effect.

Epigenetics is the collectively heritable changes in phenotype due to processes that arise independent of primary DNA sequence. Epigenetic mechanisms include DNA methylation, histone modifications and RNA interference. All of them are associated with regulation and determination of the cellular transcriptome, thereby pivotal to cell function.⁸⁹ Because epigenetic modifications are inherited across generations, but are not assayed by genotyping chips or by whole-genome sequencing,⁹⁰ it is difficult to exclude the possibility that epigenetic alterations could account for a proportion of T1D risk normally attributed to genetics.

Interestingly, accumulating evidence suggests that ncRNAs are involved in T1D pathogenesis, see above, genetic association between T1D and histone deacetylases exists,²⁰ and histone deacetylases inhibitors promote β -cell development, proliferation, differentiation and function.⁹¹ Finally, increased DNA methylation variability in T1D across different immune effector cell types has been reported.¹⁴ Nevertheless, at this time, estimates of epigenetic missing heridity are not widespread for complex diseases. However, this is a major research area to be explored to complete our understanding of T1D genetic risk.

GWAS in T1D have been focused on populations of European descent. However, the degree to which observation gained from these studies is transferable to other populations has not been extensively explored. Nevertheless, this has facilitated the success of GWAS owing to more homogeneous populations being studied.^{92–94} It is essential to perform genetic studies in non-European populations to bring medical advances from genetic studies to populations worldwide. Also characterizing risk variants beyond what can be achieved with populations of European descent alone will be crucial. The motivation for genetic studies in diverse populations is obvious as differences in disease allele frequency and LD patterns, in phenotypic prevalence, and in effect size as well as differences in rare variants exist. Several observations suggest that no single population is sufficient for fully uncovering the variants underlying T1D in all populations.

Common statistical analysis of GWAS data tests single-nucleotide polymorphisms (SNPs) individually.⁹⁵ However, single SNP analysis may be underpowered, especially for low-frequency SNPs.⁹⁶ Individual

genes, gene networks and pathways are all genetic entities that are likely to have multiple SNPs that function simultaneously to affect diseases and traits. Several novel methods for analyzing GWAS data are being developed.⁹⁷

A final restraint in GWAS is the classical case–control design. The power of GWAS to identify a true association between a SNP and trait is dependent on the phenotypic variance within the population explained by the SNP. However, to date most GWAS of T1D have identified risk loci by comparing cases versus controls independent of heterogenic disease sub-phenotypes, persisting autoantibody positivity, disease progression, for example, loss of β -cell function/mass, complication status and so on. Studies are now emerging looking at genetic risk for autoimmunity development before clinical onset,⁹⁸ for decline in β -cell function after diagnosis,⁹⁹ for persistent autoimmunity^{100,101} among other T1D-related sub-phenotypes. Intriguingly, a number of new associations are reported from these studies emphasizing that we still have a lot to learn about T1D genetics.

CONCLUSIONS

This review summarizes some of the advances and challenges in GWAS-identification of T1D-associated risk variants. GWAS have led to the identification of >60 loci associated with T1D. Importantly, these loci seem to explain most of the heritability of T1D, which is in contrast to most other complex disorders. Thus, it is timely to dissect and translate this genetic predisposition to a deeper understanding of disease biology. Current genetic understanding is being leveraged by complementary studies in epigenetics, transcriptomics, proteomics, metabolomics and lipidomics of both β cells and immune cells. Novel approaches as next-generation-sequencing of both DNA and especially RNA with the ability to identify allelic imbalance, to quantify gene expression in a transcript-specific manner, and to capture unexpected alternative splicing, truncation and post-transcriptional modification events hold great promises. The use of inducible pluripotent stem cells holds great potential for functional studies of, for example, β -cells in the context of relevant genetic risk profiles. A challenge not easily accomplished by the use of human donor pancreatic islets. It is expected that this genetic insight will transform the landscape of common complex diseases such as T1D and lead to novel treatment, preventive strategies and enable precision medicine based on genetic profiling.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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